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The Use of Polyethylene Estrogen Capsules in the Chronic Steroid Treatment of Prepubertal Female Rats (40774)[1]

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Consistently low levels of radioimmunoassayable estrogen are present just prior to puberty in the female rat (1, 2). Hence, a slow steroid-release capsule would be required to maintain physiological serum estrogen levels in young ovariectomized rats. Although Silastic capsules have been used successfully in the large animal, their release rate may be too high and therefore not suitable for chronic steroid treatment of the prepubertal animal. When implanted subcutaneously, Silastic capsules containing 17β-estradiol (E_2) alone or mixed with peanut oil (3) produced serum levels of 100–150 and 10–40 pg E_2/ml, respectively in animals weighing more than 225 g. Alzet osmotic minipumps (Alza Corp., Palo Alto, Calif.), delivering 1 μg E_2/24 hr produced serum concentrations of approximately 40 pg E_2/ml (4). Previously it has been shown that the release rate of steroids from polyethylene (PE) is remarkably less (1/1000) than that of Silastic (5). Thus, these experiments were designed to test the effectiveness of PE estrogen capsules to maintain a physiological level of serum estrogen in the ovariectomized prepubertal animal.

Materials and methods. Animals. Sprague–Dawley female rats were received from the supplier (Simonsen) when they were 23 days of age and were housed under controlled conditions of temperature and illumination. At 24 days of age bilateral ovariectomy was performed and the females were treated with E_2 in either Silastic or polyethylene capsules inserted subcutaneously (described below). At 30 days of age the animals were lightly anesthetized with ether and 1 ml of blood was obtained by jugular puncture. The animals were then sacrificed and examined for the occurrence or absence of vaginal opening, and the uterine weights recorded. Blood samples were centrifuged and the serum stored at 4°C until assayed for LH.

Estradiol capsules. Polyethylene capsules were prepared by placing a 4-mm wooden plug into one end of polyethylene tubing (0.070 in. i.d. × 0.110 in. o.d.) and sealing the exposed tip of polyethylene with heat. E_2 (Sigma) alone or mixed 1:1–1:10 with cholesterol was packed into tubing in lengths ranging from 2.5 mm to 2 cm. Another wooden plug was inserted at the open end and the polyethylene tubing heat sealed. Silastic capsules were prepared as previously described (6). Prior to insertion into the animals the capsules were washed in phosphate-buffered saline for 48 hr at 37°C. The buffer was replaced six times during the incubation period.

In vitro experiments. In order to determine the release rate of estrogen from the capsules in an *in vitro* system, prewashed capsules of various lengths were added to 5 ml of estrogen assay buffer (sodium phosphate 0.1 *M*, ph 7.0,0.1% gelatin) at 37°C, shaken in a Dubnoff Shaker, and samples (50 μl) collected at 3, 6, 9, and 24 hr, at which time old incubation medium was replaced with 5 ml of new buffer. The samples resulting from the incubation of PE capsules were added directly to 450 μl of estrogen assay buffer and subsequently assayed

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directly (without chromatography) for E_2. Aliquots resulting from the incubation of Silastic capsules were diluted further with assay buffer prior to transfer to assay tubes. In the *in vitro* system, 2.5 mm Silastic capsules were used rather than the 3-mm capsule.

LH and E_2 assays. LH concentrations were measured with the radioimmunoassay kit (A. F. Parlow) supplied by NIAMDD, using the RP-1 standard. The radioimmunoassay for E_2 has been described previously (7).

Statistical comparisons were made by Student's *t* test.

Results. The mean uterine weight in the intact animals on Day 30 was 47 mg and was reduced to 28 mg by ovariectomy on Day 24. Treatment with PE capsules of various lengths (2.5 mm–2 cm) containing 100% E_2 resulted in uterine weights ranging from 100 to 152 mg (Fig. 1), while even heavier uterine weights (182 mg) were induced by 3 mm Silastic implant treatment ($P < 0.001$ vs 2 cm PE capsule). Estrogen PE capsule lengths of 2.5 mm to 1 cm produced essentially identical uterine weights. However, reduced uterine weights were observed when animals were treated with 2.5- and 5-mm capsules diluted with various concentrations of cholesterol (Fig. 2). The 2.5-mm PE capsule diluted with 50% cholesterol induced a uterine weight comparable to uterine weights observed in the intact animals. Uterine weights similar to those found in the castrated animals resulted from treatment with 2.5-mm PE capsules diluted 1:10 with cholesterol.

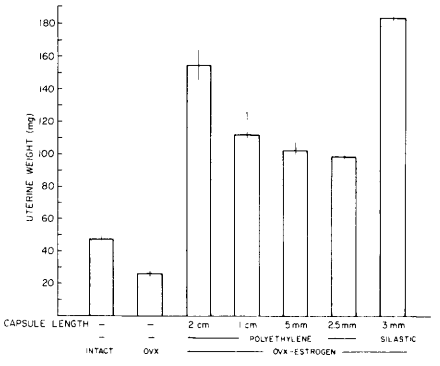

FIG. 1. Effects of polyethylene and Silastic E_2 implant treatment on uterine weights in 30-day-old, ovariectomized (OVX) female rats. Ovariectomy and estrogen treatment was initiated on Day 24. Each bar represents the mean and the vertical line the standard error.

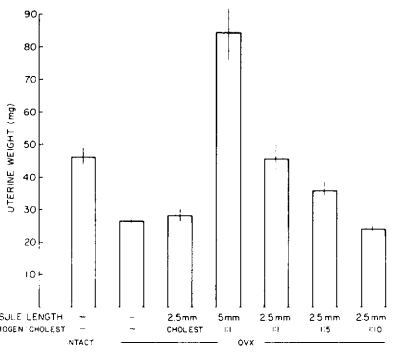

FIG. 2. Uterine weights in ovariectomized (OVX), 30-day-old female rats treated with PE capsules containing various concentrations of E_2 diluted with cholesterol (Cholest).

Neither intact nor ovariectomized animals demonstrated a vaginal opening on Day 30, but treatment with PE and Silastic capsules containing 100% estradiol resulted in a vaginal opening in all of the animals (Table I). Implantation with 2.5 and 5 mm PE capsules diluted 1:1 with cholesterol was followed by 15 and 43% of the animals exhibiting a vaginal opening, respectively. Only 25% of the intact animals had a measurable concentration of LH (in 200 μl of the serum), while all of the ovariectomized animals had elevated LH levels. Treatment with PE or Silastic capsules containing 100% estradiol completely suppressed the postcastration rise of LH in all but one animal, while 30% of the animals demonstrated a measurable LH level when implanted with 2.5- and 5-mm capsules diluted 50% with cholesterol. In the animals receiving the 5-mm implant, the LH level was essentially comparable to intact, control animals. Further dilutions with cholesterol in the 2.5-mm capsules resulted in high LH concentrations.

The release rates of E_2 from PE and Silastic capsules under *in vitro* conditions are shown in Table II. A statistically significant ($P < 0.001$) decrease in the release of E_2 from PE capsules occurred as a result

TABLE I. THE INCIDENCE OF VAGINAL OPENING AND SERUM LH CONCENTRATIONS IN ESTROGEN-TREATED 30-DAY-OLD FEMALE RATS

Treatment	No. animals	Percentage of animals exhibiting		LH (ng/ml) [a]
		Vaginal opening	Measurable LH	
Intact	16	0	25	69 ± 7
Ovariectomized	15	0	100	310 ± 10
Ovariectomized + PE capsules Estrogen only				
2 cm [b]	10	100	0	—
1 cm	5	100	20	116
5 mm	5	100	0	—
2.5 mm	5	100	0	—
Estrogen: cholesterol				
5 mm–1:1	7	43	29	48 ± 10[c,d]
2.5 mm–1:1	13	15	31	268 ± 33
2.5 mm–1:5	9	0	89	148 ± 10[d]
2.5 mm–1:10	4	0	100	280 ± 30
2.5 mm–chol.	6	0	100	301 ± 78
Ovariectomized + silastic capsules Estrogen only				
3 mm	5	100	0	—

[a] Mean ± SE of those cases in which levels were detectable.
[b] Capsule length.
[c] Not significantly different from intact animals.
[d] Significantly ($P < 0.001$) different from non-treated, ovariectomized animals.

of E_2 dilution with cholesterol. Silastic capsules containing 100% E_2 released approximately 100 times more E_2/hr than the PE capsules of equal size.

Discussion. It now appears that there is a shift in both negative and positive hypothalamic–pituitary sensitivity to estrogen when the female approaches puberty (8–10). Since this steroid is so important during the prepubertal period, we felt that it would be useful to have available an estrogen implant which could produce a constant physiological level of estrogen in the blood of ovariectomized prepubertal rats. In the present study we found that a 2.5-mm PE capsule containing 1 part E_2 to 1 part cholesterol, when implanted into an ovariectomized animal, produced a uterine weight and incidence of vaginal opening comparable to that in the intact animal. Serum LH, however, was high in those animals with a measurable LH. A larger capsule (5 mm, diluted 1:1), and therefore more estrogen, was needed to reduce the blood levels of LH to those seen in the intact animal. This may indicate that at this age, the uterus is more sensitive to the growth-stimulating effect of E_2 than the

TABLE II. THE *in vitro* RELEASE OF E_2 (ng/hr) FROM POLYETHYLENE AND SILASTIC CAPSULES

2.5 mm polyethylene			2.5 mm Silastic
1:0 [a]	1:1	1:5	1:0
1.12 ± 0.06 [b]	0.78 ± 0.06 [c]	0.32 ± 0.03[d]	100 ± 9
(5)	(5)	(5)	(6)

[a] E_2 : cholesterol.
[b] Mean ± SE.
[c] Significantly different ($P < 0.001$) from 1:0 capsule.
[d] Significantly different ($P < 0.001$) from 1:1 capsule.

hypothalamic–pituitary complex is to estrogen feedback at this time. However, it is a possibility that other steroids from the adrenal may be responsible for the differences observed, or that the constant-release capsules provide a less effective signal for LH feedback regulation when compared to the changing E_2 levels in the normal rat. In a previous study, 10 and 20 ng estradiol benzoate in corn oil/100 g body wt was effective in suppressing plasma LH in 27-day-old rats which had been ovariectomized for 2 days and treated each postoperative day (11). Although there was an estrogen dose-related decline in serum LH, no dose–response relationship existed in regard to E_2 levels. In a preliminary experiment, we also found low and variable E_2 serum levels in our capsule-implanted, prepubertal animals. In ovariectomized adults, however, 2-cm PE capsules containing 100% E_2 yielded mean serum E_2 concentrations of 6 $\pm$ 0.8 pg following 5 days of treatment (unpublished). This serum concentration was below that previously reported for Silastic capsules filled with the steroid and diluted with peanut oil (3).

Under *in vitro* conditions the 2.5-mm PE capsules released approximately 1 ng E_2/hr when filled with 100% E_2, while capsules diluted with cholesterol yielded lower E_2 release rates. On the other hand, the Silastic capsules had a 100-fold greater E_2 release than the PE capsules. Thus, these studies demonstrate that polyethylene is the polymer of choice for a capsule design when a low level of steroid release is desired. Further studies are currently in progress using the E_2 PE capsules in both young and adult female rats.

Summary. A polyethylene capsule suitable for the administration of small amounts of E_2 to young ovariectomized rats is described. A 2.5-mm PE capsule diluted 1:1 with cholesterol produced a uterine weight and incidence of vaginal opening comparable to that in the intact animal. Under *in vitro* conditions, this capsule released 0.78 ng E_2/hr, while Silastic capsules of comparable size, containing 100% E_2, produced 100 ng E_2/hr.

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